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Antienvelope Antibodies Are Protective Against GBV-C Reinfection: Evidence From the Liver Transplant Model

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An assay for the detection of antibody against the second envelope (E2) protein of GB virus type C (GBV-C) has been developed. Early reports suggested that this antibody was a marker of viral clearance, yet it is unknown whether anti-E2 is protective against further GBV-C infection. The primary aims were to determine (1) if posttransplantation immunosuppression alters the prevalence of anti-E2; and (2) if anti-E2 positivity pretransplantation protects against acquisition of GBV-C infection posttransplantation. Fifty-four recipients who underwent orthotopic liver transplantation for end-stage liver disease of nonviral etiologies were tested for GBV-C RNA using a PCR-based assay and anti-E2 antibodies by an enzyme-linked immunoassay. Anti-E2 was present in 35% and in 46% of patients pre- and posttransplantation, respectively. Anti-E2 positivity pretransplantation was strongly associated with anti-E2 positivity after transplantation ($P < 0.001$); 83% of patients with anti-E2 prior to transplantation remained anti-E2–positive after transplantation. A negative association between presence of GBV-C viremia and presence of anti-E2 was found in all patients tested either prior to or following transplantation ($P = 0.03$). Acquisition of GBV-C infection was significantly lower in patients who were anti-E2–positive prior to transplantation (2/13) compared to those who were anti-E2–negative (12/26) ($P = 0.05$). It is concluded that immunosuppression does not reduce the prevalence of anti-E2 after transplantation in those who are seroreactive prior to transplantation. Anti-E2 appears to be a neutralizing antibody whose presence at the time of liver transplantation protects against acquisition of GBV-C infection in the peritransplantation period. ***J. Med. Virol. 56:253–258, 1998.***

KEY WORDS: anti-E2; liver transplantation; protective antibodies; GB virus-C; GBV-C acquisition; immunosuppression

INTRODUCTION

A new virus of the *Flaviviridae* family was discovered in 1995 and was named GBV-C [Simons et al., 1995]. GB virus type C (GBV-C) has a single-stranded RNA genome of approximately 10 kb. A related virus, hepatitis G virus (HGV), was discovered in 1996 [Linnen et al., 1996]. HGV and GBV-C were subsequently shown to be isolates of the same virus [Linnen et al., 1996; Zuckerman, 1996]. GBV-C/HGV viremia has been reported in populations at risk for acquisition of parenterally transmitted infectious agents such as transfusion recipients [Jarvis et al., 1996; Feucht et al., 1997a, 1997b], injection drug users (IDU) [Stark et al., 1996; Feucht et al., 1997a, 1997b], and patients on hemodialysis [Masuko et al., 1996; Feucht et al., 1997a, 1997b]. GBV-C/HGV has also been reported in patients infected with other parenterally transmitted viruses such as hepatitis C virus (HCV) and hepatitis B virus (HBV) [Berenguer et al., 1996 ; Marrone et al., 1996; Feucht et al., 1997a, 1997b].

The search for serologic markers of GBV-C has focused on regions homologous to those identified for HCV because of the phylogenetic relatedness of GBV-C and HCV [Pilot-Matias, 1996]. The core (C) protein is one of the most important markers of HCV infection [Chiba et al., 1991; Nasoff et al., 1991]. However, GBV-C core protein is either "absent" or "truncated"

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[Erker et al., 1996; Leary et al., 1996; Simons et al., 1995], which explains, in part, the difficulties encountered in the development of specific and sensitive serological assays for this virus.

Antibodies against envelope proteins (anti-E2) have also been useful serological markers of HCV infection [Lok et al., 1993; Lesniewski et al., 1995]. In chronic HCV infection, the coexistence of E2/NS1 antibody and viremia suggests that the E2 antibody does not have neutralizing activity, but rather serves as a marker of active HCV replication [Yuki et al., 1996]. Recently, an antibody assay based on the E2 protein of GBV-C was developed [Dille et al., 1997; Lou et al., 1997]. Preliminary studies described GBV-C E2 antibody in association with viral clearance [Pilot-Matias et al., 1996; Hassoba et al., 1997]. However, the exact role of GBV-C E2 antibody as an immune marker and its relationship to protection against subsequent GBV-C infection are still under investigation.

GBV-C/HGV infection has commonly been found in many studies of patients undergoing liver transplantation [Feucht et al., 1997a, 1997b; Stark et al., 1997]. A prevalence of 22% and 24% was reported in pre- and posttransplantation serum samples of liver transplant recipients with HCV/HGV coinfection [Berenguer et al., 1996]. A higher posttransplantation prevalence (67%), together with an apparently high rate of viral acquisition in the posttransplantation period (53%), was reported in patients who were transplanted for cryptogenic cirrhosis and for liver disease of nonviral etiologies [Fried et al., 1997; Pessoa et al., 1997]. Feucht et al. [1997a, 1997b] also described an increased prevalence of GBV-C viremia in liver transplant recipients (6% prior to transplantation versus 45% posttransplantation), a finding supported by another group [Dickson et al., 1997]. While GBV-C/HGV infection was reported to be significantly associated with the number of blood transfusions in renal transplant recipients, we have been unable to confirm these observations in liver transplantation recipients [Pessoa et al., 1997; Stark et al., 1997]. The high prevalence of GBV-C viremia after transplantation has been hypothesized to be due to the acquisition of the virus in the peritransplantation period and/or to the reactivation of low levels of viremia under the influence of posttransplantation immunosuppression [Berenguer et al., 1996; Pessoa et al., 1997].

The prevalence of GBV-C E2 antibody in adult liver transplantation recipients and the clinical impact of detectable antibody have not been investigated previously and are the focus of this current study. This study was designed primarily to determine whether posttransplantation immunosuppression alters the prevalence of serological markers for GBV-C and whether the presence of anti-E2 is protective against subsequent GBV-C infection. A secondary aim was to determine whether the presence of anti-E2 following transplantation influenced posttransplantation outcomes such as death, need for retransplantation, development of posttransplantation hepatitis, and episodes of rejection.

MATERIALS AND METHODS

Study Population

Fifty-four adult patients who had undergone liver transplantation for nonviral etiologies at the University of California, San Francisco, in the period from 1988 to 1996 were included. Patients who were infected with HCV and/or HBV before transplantation were excluded (anti-HCV or HCV RNA-positive, HBsAg or anti-HBc–positive). Clinical data were obtained by reviewing patients' charts. Data evaluated included age, gender, ethnicity, number of different blood donors per patient (measured during the initial hospitalization), rate of retransplantation, and death. Ethnicity was classified as: (1) Caucasian, (2) Hispanic, (3) African American, (4) Asian, and (5) others. Pre- and posttransplantation sera were tested for GBV-C RNA, and anti-E2. Pretransplantation serum was obtained on the day of liver transplantation and posttransplantation samples were obtained at a mean of 35 months posttransplantation (range = 1–87 months). Liver tests (alanine transminase [ALT], aspartate transminase [AST], and total bilirubin) were measured in all patients concurrent or within 4 weeks of GBV-C RNA and anti-E2 measurements posttransplantation. All patients gave informed consent under a protocol approved by the Institutional Review Board of the University of California. None of the donors (organ or blood) were tested for either GBV-C RNA or anti-E2.

Definitions

Active GBV-C infection was defined by the presence of GBV-C RNA with or without the presence of anti-E2. Past exposure to GBV-C infection was defined as the presence of anti-E2 with or without the presence of GBV-C RNA.

Laboratory Methods

Paired pre- and posttransplantation serum samples were available in 52/54 patients.

GBV-C detection. The GBV-C RNA genome was extracted from 25 μL serum using 280 μL buffer AVL/carrier RNA (QIA amp Viral RNA kit) as previously described [Hassoba et al., 1997].

Viral RNA was amplified using polymerase chain reaction (Abbott LCx-GBV-C Assay, Abbott Laboratories, Abbott Park, IL), which uses primers from the conserved 5′ ntr region of the GBV-C genome [Marshall et al., 1998]. The amplification product was detected in an LCx analyzer using a microparticle enzyme immunoassay (MEIA) as described by Hassoba et al. [1997] and Marshall et al. [1998].

GBV-C E2 antibody detection. The immunoassay for detection of human antibody elicited against GBV-C was carried out at Abbott Laboratories. Briefly, a glycosylated form of GBV-C E2 protein was purified and used as an antigenic target for detection of human anti-GBV-C [Dille et al., 1997]. Serum samples were

tested by an indirect immunoassay, which employed the E2 protein on a solid phase to capture antibody from human serum or plasma, followed by the addition of an enzyme-conjugated antihuman antibody for color development. Positive or gray-zone results were confirmed using a sandwich enzyme-linked immunoassay (EIA) format in which samples were reacted with E2-coated polystyrene beads, followed by addition of solution-phase biotinylated E2. Detection of antibody-captured biotinylated E2 was accomplished by addition of enzyme-conjugated antibiotin antibody [Lou et al., 1997].

Histological Assessment

Protocol liver biopsy specimens were undertaken weekly in the early posttransplantation period and annually thereafter in the majority of patients. Liver biopsies were used to assess the presence of hepatitis at any time after liver transplantation, and the number of rejection episodes per patient. In assessment of posttransplantation hepatitis, only 36 patients had liver biopsies that could be reviewed for the presence/absence of posttransplantation hepatitis. The remaining 20 patients had conditions such as biliary obstruction, cytomegalovirus hepatitis, or rejection, which did not allow accurate assessment of hepatitis.

Statistical Analyses

Values are expressed as median, range, and percentages as indicated. Categorical variables were assessed by χ^2 or Fisher's exact test. Continuous variables such as age, alanine transaminase, aspartate transaminase, total bilirubin levels, number of blood donors per patient, number of histological rejection episodes per patient, and duration of posttransplantation follow-up were compared using Mann-Whitney U test (nonparametric test).

RESULTS

Study Population Characteristics

Of the 54 study patients, the male-to-female ratio was 21:33 and median age at the time of transplantation was 52 (range 34–68). Thirty-five (65%) patients were Caucasian, 6 (11%) Hispanic, 2 (4%) Asian, 2 (4%) African American, 1 (2%) Middle Eastern, 2 (4%) other ethnicities, and 6 (11%) unknown. Pathological diagnoses at the time of transplantation were: alcoholic liver disease, 18 (33%); primary biliary cirrhosis, 14 (26%); primary sclerosing cholangitis, 10 (19%); cryptogenic cirrhosis, 4 (7%); hemochromatosis, 3 (6%); autoimmune hepatitis, 2 (4%); and others, 3 (6%). Five (9%) patients required retransplantation, 2 (4%) patients acquired hepatitis C infection posttransplantation, and 6 (12%) died during follow-up. Although almost all of the patients lacked a history of known parenteral exposure prior to transplantation, 89% (48/54) received blood transfusion in the peritransplantation period.

TABLE I. Relationship Between GBV-C E2 Antibody Pre- and Posttransplantation[a]

	Post-OLT anti-E2 (+)	Post-OLT anti-E2 (–)
Pre-OLT anti-E2 (+) (n = 18)	15 (83%)	3 (17%)
Pre-OLT anti-E2 (–) (n = 34)	9 (26%)	25 (74%)

[a]n = 52; P <0.001 (χ^2 test); OLT = orthotopic liver transplantation.

Prevalence of Anti-GBV-C E2

The prevalence of GBV-C E2 antibody in our patients was 35% (18/52) and 46% (24/52) prior to and following transplantation, respectively (difference in prevalence 11%, 95%; CI: 7%, 30%; P = NS). Detectable anti-E2 pretransplantation was highly associated with anti-E2 posttransplantation ($P < 0.001$) (Table I). Of 18 patients serologically positive prior to transplantation, 15 (83%) remained positive while only three patients lost antibody after transplantation. Antibody loss was unrelated to duration of follow-up ($P = 0.61$, Mann-Whitney U test). Of the 34 patients who were negative for anti-E2 before transplantation, 25 (74%) remained anti-E2–negative after transplantation (Table I).

The presence of anti-E2 posttransplantation was unrelated to factors that may influence posttransplantation outcomes, such as age, gender, biochemical liver tests, the presence or absence of posttransplantation hepatitis, posttransplantation acquisition of HCV infection, number of donor exposures, number of rejection episodes, duration of follow-up, retransplantation, and/or death (Table II). None of the patients acquired HBV infection after transplantation.

Prevalence of GBV-C RNA

GBV-C RNA was detected in pre- and posttransplantation serum samples of 30% (16/54) and 41% (22/54) of patients, respectively ($P = 0.13$, χ^2 *test*). Forty-four percent (7/16) of patients with active infection prior to transplantation lost GBV-C RNA following transplantation, while 34% (13/38) of patients without active infection prior to transplantation had detectable GBV-C viremia after transplantation (data not shown).

Pretransplantation GBV-C viremia was unrelated to age ($P = 0.58$, Mann-Whitney U test), gender ($P = 0.28$, χ^2 test), ethnicity (Caucasian versus others, $P = 0.82$, χ^2 test), or pretransplantation pathological diagnosis ($P = 0.27$, χ^2 test). Posttransplantation GBV-C viremia was unrelated to ALT level ($P = 0.72$, Mann-Whitney U test), number of donor exposures per patient ($P = 0.39$, Mann-Whitney U test), acquisition of hepatitis C ($P = 0.67$, Fischer's exact test), presence of histological hepatitis ($P = 0.52$, Fischer's exact test), retransplantation ($P = 0.31$, Fischer's exact test), or death ($P = 0.19$, Fischer's exact test).

Relationship Between GBV-C E2 Antibody and GBV-C Viremia

A significant negative association was demonstrated between anti-E2 and GBV-C viremia ($P = 0.03$) (Table III). This negative association was more predominant

TABLE II. Characteristics of Patients With and Without Anti-E2 Posttransplantation[a]

Clinical features	Anti-E2 (+) (n = 24)	Anti-E2 (–) (n = 30)	*P* value
Age median (range)	53 (34–68)	52 (36–62)	0.46[d]
Gender (M:F)	13:11	8:22	0.11[b]
ALT (IU/L) n = 37 (XULN) median (range)	0.45 (0.1–0.8)	0.91 (0.2–3.3)	0.75[d]
AST (IU/L) n = 50 (XULN) median (range)	0.68 (0.3–2.2)	1.30 (0.2–3.7)	0.20[d]
Total bilirubin (mg/dL) n = 50	0.73 (0.3–1.4)	1.00 (0.2–5.7)	0.30[d]
Post-OLT hepatitis (n = 36)			0.37[c]
Yes	2	1	
No	13	20	
Acquired HCV (n = 50)			0.69[c]
Yes	1	1	
No	21	27	
Number of donors per patient (n = 50) median (range)	50 (17–213)	44 (15–177)	0.42[d]
Number of rejection episodes (n = 51) median (range)	0 (0–5)	0 (0–4)	0.40[d]
Duration of anti-E2 F/U (months) median (range)	24.3 (0.46–87.8)	33 (8.9–76.5)	0.90[d]
Retransplantation (n = 50)			0.44[c]
Yes	1	0	
No	21	28	
Post-OLT death (n = 52)			0.75[b]
Yes	3	3	
No	19	25	

[a]n = 54; OLT = orthotopic liver transplantation; ALT = alanine transaminase; AST = aspartate transaminase; F/U = follow-up; XULN = times upper limit normal.
[b]χ^2 test.
[c]Fisher's exact test.
[d]Mann–Whitney U test.

TABLE III. Association Between Anti-E2 and GBV-C RNA in All Samples Tested[a]

	GBV-C RNA (+)	GBV-C RNA (–)
Anti-E2 (+) (n = 42)	9 (8%)	33 (31%)
Anti-E2 (–) (n = 66)	28 (26%)	38 (35%)

[a]Total samples tested = 108; $P = 0.03$ (χ^2 test).

in the posttransplantation period (data not shown). Simultaneous presence of anti-E2 and GBV-C RNA in patients samples was uncommon. Of 70 patient samples positive for past or current GBV-C infection (anti-E2 and/or GBV-C RNA), only nine (13%) were simultaneously positive for antibody and RNA. In contrast, E2 antibody and GBV-C RNA was mutually exclusive (one but not both tests were positive) in 61/70 (87%) of samples. Thirty-eight samples negative for both anti-E2 and GBV-C RNA were excluded from this analysis.

Pretransplantation Anti-E2 and Acquisition of Posttransplantation GBV-C Infection

Acquisition of posttransplantation GBV-C infection was compared between patients with and without anti-E2 prior to transplantation (n = 39) (Table IV). All patients lacked GBV-C RNA prior to transplantation. The presence of anti-E2 prior to transplantation protected against acquisition of GBV-C RNA posttransplantation ($P = 0.05$). Only 2/13 (15%) anti-E2–positive patients pretransplantation became infected with GBV-C after transplantation. In contrast, 12/26 (46%) of anti-E2–negative patients pretransplantation had detectable GBV-C RNA after transplantation. Ten (83%) of 12 patients who acquired GBV-C infection remained anti-E2–negative posttransplantation. The number of blood donor exposures and rejection episodes per patient was not associated with posttransplantation acquisition of GBV-C RNA ($P = 0.31$ and 0.64, respectively, Mann-Whitney U-test, data not shown).

TABLE IV. Relationship Between anti-E2 Pretransplantation and Acquisition of GBV-C RNA Posttransplantation in Patients Without Active GBV-C Infection Prior to Transplantation[a]

	Post-OLT GBV-C RNA (+)	Post-OLT GBV-C RNA (–)
Pre-OLT Anti-E2 (+) (n = 13)	2 (15%)	11 (85%)
Pre-OLT Anti-E2 (–) (n = 26)	12 (46%)[b]	14 (54%)

[a]n = 39; $P = 0.05$ (Fisher's exact test).
[b]10/12 patients remained anti-E2–negative.

DISCUSSION

GBV-C is a human virus that has been described in a number of patient population with or without liver disease. Clinical significance of either prior exposure to GBV-C or ongoing infection with this virus is under investigation, but multiple studies appear to suggest that GBV-C/HGV is neither hepatotropic, nor associated epidemiologically with acute or chronic liver disease [Alter et al., 1997a, 1997b; Pessoa et al., 1998]. Moreover, prior studies have failed to find an association between GBV-C infection and severity of liver disease either in transplanted or nontransplanted patient populations [Berenguer et al., 1996; Martinot et al., 1997]. To date no serological markers of chronicity have been identified, and diagnosis of chronic infection

has relied on detection of viral RNA by molecular techniques. On the other hand, an antibody against GBV-C E2 protein has been described as a marker of viral clearance either in association with other viruses (HCV, HBV, or HIV) or in association with different risk factors for parenteral exposure, i.e., injection drug use, hemophilia, dialysis, or multiple transfusion [Pilot-Matias et al., 1996; Hassoba et al., 1997].

To our knowledge this is the first report to describe the prevalence and clinical significance of anti-GBV-C E2 in a liver transplant population. In the current study the prevalence of anti-E2 in pretransplantation serum samples in our study (35%) is comparable to that recently described in renal transplant recipients (40%), and in those with a history of injection drug use (41%) [Stark et al., 1997; Tacke et al., 1997]. This prevalence is lower than that previously observed in our study on Egyptian patients with chronic HCV (58%) [Hassoba et al., 1997], but higher than that in volunteer blood donors (9.1%) [Tacke et al., 1997]. The high prevalence of this parenterally transmitted virus in our patients undergoing liver transplantation is currently unexplained, since the majority lacked a history of parenteral risk factors other than transfusion (89% of our population received blood transfusion in the peritransplantation period, but none of these units of blood were tested for either the virus or its antibody). Our study does confirm previous investigations in other patient populations describing anti-E2 as a marker of viral clearance [Pilot-Matias et al., 1996; Hassoba et al., 1997; Tacke et al., 1997], since few patients with detectable antibody also had detectable viral RNA.

The detection of GBV-C RNA and GBV-C E2 antibody are usually mutually exclusive [Pilot-Mathias et al., 1996; Tacke et al., 1997]. However, simultaneous presence of both GBV-C RNA and anti-GBV-C E2 has been observed in many studies (2/458 [Gutierrez et al., 1997]; 10/235 [Feucht et al., 1997a, 1997b]; 2/221 [Stark et al., 1997]; 4/99 [Tacke et al., 1997]). In this report, 13% (9/70) of patients' samples were obtained at the point where both anti-E2 and viral RNA were detectable. This is likely to reflect the period in which the antibody begins to clear the virus. Dille et al. [1997] in a set of serial specimens from four patients has shown that the development of anti-E2 corresponds to a loss of detectable GBV-C virus. During the course of examination, three of four subjects were dually reactive for GBV-C RNA and GBV-C E2 antibody in at least one of the serial specimens tested. These dually positive specimens were found during the transition between viremia and anti-E2 positivity/viral clearance. [Dille et al., 1997]. Other possible explanations include passive antibody transfer (all our patients have a history of blood transfusion) and false-positive EIA results.

We have shown that the presence of anti-E2 prior to transplantation is associated with a relatively low rate (15%) of posttransplantation GBV-C infection compared to the threefold higher rate (46%) in anti-E2–negative (pretransplant) patients. These data are highly indicative of a protective effect of antibody against subsequent infection. Viral acquisition in anti-E2–negative patients was unrelated to factors typically implicated in posttransplantation viral acquisition such as high number of blood donor exposures and the number of rejection episodes with subsequent higher doses of immunosuppression that could unmask unnoticed or early infection. However, since we were unable to test either the blood or organ donors for the presence of active GBV-C infection, it is possible that patients who acquired GBV-C infection were more commonly transfused with infected units of blood than those who remained GBV-C RNA-negative. Similar transfusion requirements in those who did and those who did not acquire GBV-C suggest that this latter explanation is unlikely.

Posttransplantation immune suppression apparently had only a minor effect on the prevalence of anti-E2 in patients who were anti-E2–positive prior to transplantation, since 83% of patients with anti-E2 prior to transplantation remained anti-E2–positive after surgery. These results are consistent with a study that evaluated the role of posttransplantation immunosuppression on the antibody response to HCV infection [Hsu et al., 1994]. In the latter study, 94% of patients with anticore pretransplantation remained antibody-positive posttransplantation, although a fourfold decrease in antibody titer was noted. The current study was not designed to quantitate anti-E2 titers so that we cannot comment on the effect of immunosuppression on the strength of the serological response. In the study of HCV, acquisition of infection in the peritransplantation period was associated with an impaired serological response to virus since only 25% of patients who acquired HCV had detectable anti-HCV core antibody [Hsu et al., 1994]. This study concluded that current serological assays are unreliable in assessing the antibody response to acquired HCV infection after transplantation. The same is also true for GBV-C infection since only 2/13 (3.7%) of those with acquired GBV-C were seroreactive for anti-E2 posttransplantation. Thus for both viruses, reliance on serological assays for diagnosis of acquired infection in these immune-compromised individuals appears problematic.

It is concluded that (1) posttransplantation immunosuppression appears to have little effect on the prevalence of anti-E2; (2) anti-E2 is a marker of viral clearance; and (3) anti-E2 protects against subsequent GBV-C infection in adult patients undergoing liver transplantation, since acquisition of the virus was uncommon in the presence of anti-E2 pretransplantation. Thus antienvelope antibodies are not only a marker of prior exposure to GBV-C infection, but also a marker of protective immunity.

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